COMMENTS ON THE NATIONAL TOXICOLOGY PROGRAMME (NTP)

DRAFT REPORT ON CARCINOGENS:

SUBSTANCE PROFILE FOR GLASS WOOL FIBRES (RESPIRABLE) AS A CLASS

Professor Kenneth Donaldson BSc PhD DSc FIBiol FRCPath FFOM

Independent Consultant

and

Professor of Respiratory Toxicology, University of Edinburgh

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I have been asked by the North American Insulation Manufacturers Association (NAIMA) to provide comments on the "Draft report on carcinogens: substance profile for glass wool fibres (respirable) as a class". My comments are confined to addressing the utility of *in vitro* tests to classify fibres as possibly carcinogenic, as this is my own special field of expertise.

Qualifications

I am Professor of Respiratory Toxicology at the University of Edinburgh and an independent consultant on pulmonary toxicology of particles and fibres. I am a Fellow of the Institute of Biology, a Fellow of the Royal College of Pathologists and a Fellow of the Faculty of Occupational Medicine by research on particle toxicology. I hold the degrees of BSc (Hons – 1st Class) from the University of Stirling in Biology (1978), and a PhD (1982) and DSc (1998) from the University of Edinburgh. I have carried out 30 years of research into the harmful effects of inhaled particles including various types of naturally-occurring and man-made mineral fibres. I have published more than 300 papers and reviews and book chapters on particle toxicology, many of them concerning fibre toxicology. I have edited a major textbook on particle toxicology of the lung and am founding Editor in Chief of the Journal Particle and Fibre Toxicology. I have lectured extensively in undergraduate general toxicology and postgraduate toxicology specialising in lung toxicology of particles and fibres and I have been invited to speak and Chair at numerous conferences on particle and fibre toxicology. I was employed by the Institute of Occupational Medicine as Head scientist from 1972-1992, by Napier University, Edinburgh as a Professor of Pathobiology 1992-2002, and the University of Edinburgh as Professor of Respiratory Toxicology from 2002 to the present. I sit or have sat on numerous UK Government, European and US committees that consider various strategic and regulatory aspects of the toxicology of inhaled particles and fibres.

As discussed in the relevant section of the Draft Substance Profile report entitled 'Fibre properties and mechanisms related to carcinogenicity' (page 4) early studies indeed '. . . demonstrated a relationship between tumor incidence and fiber size or shape . . .' and that '. . . Fiber dimensions and durability also were found to be important determinants of tumorigenicity' (page 4). The report then goes on to describe at length the key modifying role of biopersistence on long fibres pathogenicity – 36 lines on pages 4 and 5.

In summary, many high quality animal studies persuasively show that long biopersistent fibres are the effective dose for fibrosis and cancer in laboratory animals, while long non-biopersistent fibres have much reduced, or no, carcinogenic potential [1-5]. The underlying mechanism concerns the fact that long biopersistent fibres can retain their structural integrity over the time required to migrate to the interstitium and the pleura. The presence of long biopersistent fibres at these sites then leads to the induction of persistent inflammation, fibrosis and genotoxic effects [6-9]. This in turn causes fibrosis and carcinoma in the lungs, and fibrosis and mesothelioma at the pleura. In contrast, long, non-biopersistent fibres that deposit in the lung undergo dissolution and breakage to less harmful short fibres that are cleared from the airspaces or pleura and so do not cause these effects. Therefore the modifying effect of biopersistence on the effective dose of long fibres is absolutely paramount in determining whether a fibre exposure is pathogenic or not.

The biopersistence is commonly expressed as the average time that a long fibre persists in the lungs, known as the retention half-time (or clearance half-time). This differs between fibre types but the half-times, that is the time for depletion to 50% of the long (>20 μm) fibres, ranges from 2.4 days to 85 days for various wools [10]. In other words between 2.4 and 85 days residence in the lung is required for

half of the long fibres to be dissolved or, as is more likely, weakened sufficiently that they break and become shortened and, in effect, made part of the non-effective dose or cleared.

Despite having given over 40 lines to describing the key role of biopersistence, the Draft Report then gives 30 lines to describe *in vitro* bioassay studies demonstrating various endpoints such as cytotoxicity, pro-inflammatory effects and genotoxic effects that are in general fibre length dependent – long fibres being more potent that short fibres. However this latter section disregards the key role of biopersistence and the obvious disconnect between the timescale for such *in vitro* assays (24 hours – 72 hours at the most) and the clearance half-times of long glass wool fibres (2 – 85 days) due to their variation in biopersistence. Clearly, even if the conditions in an *in vitro* cell culture genotoxicity assay were to mimic the conditions in the lungs that lead to dissolution of long fibres, the timescale of *in vitro* assays are only a fraction of the retention half-time of the fastest-dissolving fibres, *i.e.*, for the great majority of glass wools the retention half-time greatly exceeds the length of an *in vitro* genotoxicity test. Therefore biopersistence, which is a key modifier of the carcinogenicity of non-biopersistent long fibres *in vivo,* cannot play a role in such *in vitro* assays, inevitably leading to <u>false positives in *in vitro* genotoxicity tests of non-biopersistent long fibres such as insulation glass wools</u>.

The mechanism of carcinogenicity of fibres also involves chronic inflammation as an indirect mechanism of genotoxicity via leukocyte–derived oxidants [11] and the same argument can be made regarding false positivity in short term assays that demonstrate pro-inflammogenic effects of long non-biopersistent fibres.

Several competent authorities have expressed similar concerns regarding the use of short-term assays that do not take account of biopersistence.

1) The IARC 2002 Monograph on synthetic vitreous fibers (SVF) [12] reported that:-

'....4.3 Toxic effects in experimental systems

This section covers selected toxic effects of fibres in experimental systems that are believed to be potentially important in relation to the carcinogenic process. These endpoints include in-vivo effects such as inflammation and fibrosis, as well as selected in-vitro assessments including cytotoxicity, oxidant production and alterations to the cell cycle including proliferation and apoptosis. Genetic toxicology end-points are reviewed in section 4.5. It is important to appreciate the degree to which biopersistence plays a role in the different studies and end-points under review, as this property of fibres is thought to be critical in determining chronic toxicity and carcinogenic outcome in humans and in experimental animal systems. In-vitro assays are invariably short-term (i.e., from hours to days), and the effect of fibre durability is unlikely to be detected in such assays. [The Working Group noted that endotoxin is a potent environmental contaminant and its presence in fibre samples could enhance their ability to cause acute inflammation. The presence of endotoxin or the steps taken to inactivate it, were not always reported.] Therefore, short-term tests could give a misleading impression of possible long-term biological effects. This will most likely become manifest as a false-positive result in an in-vitro assay for long, non-biopersistent fibres. For a non-biopersistent fibre, the

effects seen in vitro may apply only to the time interval in vivo before the fibre begins to undergo dissolution or breakage. In contrast, a durable fibre may show the much more slowly and is more likely to give rise to pathological change . . . .'

The IARC Monograph also noted on page 337 in summarizing the genetic effects of synthetic vitreous fibers:

"A major gap in the current database is the absence of any studies that correlate genotoxic endpoints with the pathogenic effects of man made vitreous fibres in the same experimental animal system."

2) Another report was the product of the joint efforts of the members of an expert working group organized and convened by the International Life Sciences Institute Risk Science Institute [13] (page 527).

'. . . Comment. There are several issues that limit the usefulness of in vitro tests for toxicity screening of fibers. For example, short-term in vitro assays of biological activity cannot allow for differences in biopersistence of fibers, and as a result, some nonbiopersistent fibers that are not pathogenic in vivo are positive in short-term in vitro tests (Hesterberg et al., 1983; Ye et al., 1999). In vitro cellular assays, in fact, have several technical limitations:

1. High doses of fibers are used to obtain a positive response; it is difficult to extrapolate from these high-dose, short-term exposures to low-dose, chronic exposures in vivo.

2. Fiber dose in cellular assays is often expressed in terms of mass of fibers rather than numbers of fibers, creating a major problem in relating in vitro to in vivo dose. In fact, number of long (>20 µm) fibers is a better dose metric to use in comparing potency between fiber types. Number of long fibers per cell is the optimal expression of dose, but number of long fibers per unit surface area of the culture dish is an acceptable alternative.

3. In vitro endpoints (e.g., release of inflammatory mediators, activation of transcription factors, induction of cell proliferation or apoptosis) are measured after a few hours or days, while in vivo responses to biopersistent fibers are sustained over several weeks or months. These endpoints have not been validated as screening assays that are predictive of long-term pathological effects in vivo.

Conclusion In summary, a number of authorities are in agreement with my own experience regarding the shortcomings of short-term *in vitro* assays for predicting genotoxicity of long non-biopersistent fibres. In contrast to the usefulness of such short-term genotoxicity studies for many chemicals, their utility and the significance of reported findings, are subject to a number of recognized limitations with regard to SVFs. In short they are liable to produce false positive outcomes for long non-biopersistent

fibres such as insulation glass wools, because the key property of biopersistence, which modifies the toxicity of long fibres, is not realised in such short-term assays.

Kenneth Donaldson

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